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HEMORRHAGE-INDUCED VASCULAR HYPOREACTIVITY TO NOREPINEPHRINE IN SELECT VASCULATURES OF RATS AND THE ROLES OF NITRIC OXIDE AND ENDOTHELIN

Liang-ming Liu,* John A. Ward,† and Michael A. Dubick*

**U.S. Army Institute of Surgical Research and †Brooke Army Medical Center, San Antonio, Texas 78234-6315*

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ABSTRACT—Hemorrhage-induced vascular hyporeactivity to norepinephrine (NE) and the possible effector roles of nitric oxide (NO) and endothelin (ET) were investigated in different vascular beds of rats. Under urethane anesthesia, rats (n = 7 per group) were hemorrhaged to a mean arterial pressure (MAP) of 50 mm Hg for 60 min. A group of rats was pretreated with either NG-nitro-L-arginine methyl ester (10 mg/kg), an NO synthase inhibitor, or PD142893 (0.1 mg/kg), an ET receptor antagonist 15 min before the end of the hypotensive period. Operated, euvolemic rats served as controls. The responses of MAP and the blood flow of the superior mesenteric (SMA), celiac (CA), left renal (LRA), and left femoral arteries (LFA) to NE (3 μg/kg, i.v.) were measured at baseline (prehemorrhage), at the end of the hypotensive period (0 h), and at 1, 2, and 4 h after the end of the hypotensive period. The pressor responses to NE on MAP at 0, 1, 2, and 4 h in the 60-min hemorrhage groups were reduced to 45.9%, 37.8%, 29.2%, 18.4% of baseline pressor response, respectively. At these same times, the fall in blood flow in response to NE in SMA, CA, LRA, and LFA was significantly blunted ($P < 0.01$). This loss of responsiveness in CA and LFA was more severe than in SMA and LRA ($P < 0.05$-$P < 0.01$). Pretreatment with L-NAME or PD142893 significantly improved the pressor response of MAP and the blood flow responses of the four arteries to NE ($P < 0.01$). Hypotension at 50 mm Hg for 60 min resulted in an apparent loss of vascular reactivity to NE, and the four vasculatures studies were not affected to the same extent. In addition, NO and ET appear to contribute to the loss of vascular reactivity in different vasculatures in hemorrhagic shock.

KEYWORDS—Vascular reactivity, blood flow, nitric oxide synthase inhibitor, endothelin receptor antagonist

INTRODUCTION

Previous studies have reported that after severe trauma or shock, or associated with the systemic inflammatory response syndrome (SIRS) and multiple organ dysfunction syndrome (MODS), the vascular reactivity to vasoconstrictors and vasodilators is greatly reduced (1–5). This reduced vascular reactivity may play an important role in the incidence, development, and outcome of the shock state and may interfere with the therapy of shock, especially with the application of vasoactive agents (6). Most of these studies concentrated on the change of overall vascular reactivity *in vivo* by observing changes in mean arterial pressure (MAP), or they examined vascular reactivity of a single blood vessel *in vitro*, such as aorta, pulmonary vessel, renal artery, cerebral vessels, or coronary artery (2, 7, 8). Of the research performed in this field, none has investigated vascular reactivity of different vasculatures at the same time after shock, sepsis, SIRS, or MODS.

Many factors, including desensitized adrenoceptors (9), nitric oxide (NO) (3, 5, 6, 10–13), endogenous opioid peptides such as beta-endorphin (14), and inflammatory cytokines such as tumor necrosis factor-α (TNF-α) (15, 16) and interleukin (IL)-1 (17) have been proposed to interfere with the vascular reactivity during shock. Among them, the role of NO in vascular hyporeactivity has been well documented, but whether the effect varies among different vascular beds remains unclear. Endothelin, a peptide with vasoconstrictor properties, also has been implicated in the pathophysiology and pathogenesis of circulatory shock, but no study has investigated its involvement in vascular hyporesponsiveness during circulatory shock. Only a few articles have reported that it is probably involved in the changes of vascular reactivity during ischemia–reperfusion (18, 19), normal physiology (20), or some disease states (21, 22).

The aims of the present study were 3-fold. The first phase determined whether a chosen duration of hemorrhagic shock in rats would induce a time-dependent effect on vascular hyporeactivity. Second, the studies determined whether hemorrhagic shock-induced vascular hyporeactivity differed among select vasculatures. Third, the studies evaluated whether NO and endothelin (ET) could have independently identifiable roles in hemorrhagic shock–induced vascular hyporeactivity in different vasculatures. To achieve these goals, the celiac (CA) and left renal arteries (LRA), representing supplies to solid organs, superior mesenteric artery (SMA), mainly supplying a hollow organ, and left femoral artery (LFA), for skeletal muscle, were selected for examination. As mentioned, previous investigations of vascular responsiveness of specific vessels were performed *in vitro* (2, 5, 7, 8). To our knowledge, this is the first study to investigate vascular reactivity *in vivo* using blood flow in different vascular beds. The methodology used to determine vascular responsiveness *in vivo* was adapted from well-established protocols that used changes in MAP as a means of estimating overall vascular responsiveness to a vasoconstrictor (23, 24).

MATERIALS AND METHODS

Instrumentation

This study was approved by the Research Council and by the Animal Care and Use Committee of the U.S. Army Institute of Surgical Research. The experiments

Address reprint requests to Dr. Michael A Dubick, 3400 Rawley E. Chambers Avenue, U.S. Army Institute of Surgical Research, Fort Sam, Houston, TX 78234-6315.

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were performed in adherence to the National Institutes of Health Guidelines on the Use of Laboratory Animals. Forty-two male Sprague-Dawley rats (Harlan, Indianapolis, IN), weighing 410 ± 34 g, were fasted 12 h but allowed water *ad libitum* before the experiment. On the day of experiment, rats were first anesthetized with 2% to 3% isoflurane and the right femoral artery and vein and right carotid artery were catheterized with PE tubing (outer diameter 0.965 mm, inner diameter 0.58 mm) for monitoring the MAP, for administering norepinephrine (NE), and for bleeding. To prevent clot formation within these cannulae, the tubing was filled with normal saline containing 30 units/mL of heparin and all attempts were made to minimize the amount of heparin that entered the body. The body temperature of the rats was maintained at 37°C with a heating pad.

A laparotomy was performed and the SMA, CA, and LRA were located and isolated, and fat and connective tissue were carefully cleaned off the vessels for proper acoustic coupling. A 0.7 V-series flow probe (Transonic Systems Inc, Ithaca, NY) was mounted around the SMA whereas 0.5 V-series probes were mounted around the CA and LRA. To optimize recordings over the entire experimental period, the vessels were nestled in the bottom of the V reflector, lubricating jelly was placed in the probe's acoustic window as couplant, and the flow probes were maintained with a proper stand. The LFA was then exposed, isolated, and a 0.5 V-series flow probe placed. All probes were connected to a Transonic flowmeter (Transonic Systems Inc) for monitoring the blood flow of these arteries. After instrumentation, rats were allowed to equilibrate for 20 to 30 min.

Experimental protocol

All hemorrhage experiments were performed under urethane anesthesia (900 mg/kg, i.p.). In Phase I, the effects of 60- or 90-min hemorrhage were investigated to define the parameters of vascular reactivity and to select the best model for the Phase II experiments. The Phase II experiments investigated the roles of NO and ET on shock-induced vascular responses to NE in four different vasculatures.

In Phase I, 21 operated and instrumented rats were randomized to three groups (n = 7/group): sham hemorrhage, 60-min hemorrhage, or 90-min hemorrhage. After baseline response measurements, rats were hemorrhaged rapidly (within 10 min) from the right carotid catheter until the MAP dropped to 50 mm Hg and then maintained at this level by additional blood removal as necessary, for either 60 or 90 min. MAP, blood flow of SMA, CA, LRA and LFA, and their responses to a vasoconstrictor (NE, 3 μg/kg bolus i.v. infusion (23, 24) were observed at 0, 1, 2, and 4 h after the end of the hypotensive period and in the sham controls. Shed blood in each group was reinfused over 10 min at the end of the hypotensive period after the time 0 measurement. MAP was monitored by a DigiMed blood pressure analyzer (MicroMed, Inc, Lexington, KY). Blood flow was measured with a Transonic T206 flowmeter (Transonic System Inc), and recorded with a DATAQ DI-220 data acquisition system (DATAQ Instruments, Akron, OH). The maximum increase in MAP after NE infusion generally reflected the systemic vascular reactivity (23, 24), and this increase at baseline was considered the 100% response. Similarly, the maximal fall in blood flow of each artery after NE administration was taken to reflect the contractile response of each artery to NE. Thus, the change in vascular reactivity after NE administration was presented as the relative change of blood flow before and after NE administration according to the following formula:

$$\text{The relative change in blood flow after NE} = (A - B)/A$$

Where A = blood flow before NE administration and B = the lowest blood flow after NE administration. The result of this calculation at baseline was assigned a value of 100%. The relative change in blood flow at subsequent experimental times was expressed as a percentage of the baseline change.

At the end of the experimental period, surviving rats were euthanatized with a pentobarbital-based euthanasia solution (Sleepaway®; 2 mL, i.v.; Fort Dodge, IA) administered through the femoral vein catheter. The duration of hemorrhage, which reduced vascular reactivity by at least 30% in any of the observed vascular beds and during which most of the rats survived 4 h, was used as the model for the Phase II study.

Based on the results of Phase I, a 60-min hemorrhage was used in Phase II. Twenty-one rats (n = 7/group) were randomized to hemorrhage, hemorrhage + NG-nitro-L-arginine methyl ester (L-NAME, NO synthase inhibitor, Sigma Chemical Co., St Louis, MO), and hemorrhage + PD142893 (nonselective ET receptor antagonist, Sigma Chemical Co.). Hemorrhage procedures were conducted as described above. L-NAME (10 mg/kg, i.v.) (25) or PD142893 (0.1 mg/kg, i.v.) (26) was administered 15 min before the end of the hypotensive period in their respective groups. The sham hemorrhage control group was the same as in Phase I. The MAP, blood flow of SMA, CA, LRA and LFA, and their responses to NE (3 μg/kg, bolus i.v. injection) were observed at the same time points as in Phase I. Measurements of MAP and blood flow were conducted as described above. Other procedures were the same as in Phase I.

Histology

After euthanasia, the four vessels examined in this study were removed from certain animals and fixed in buffered formalin according to standard practice. Hematoxylin and eosin staining of the four vessels was performed and analyzed by light microscopy to determine effects of the anesthesia on integrity of the endothelium, and to evaluate whether the observed vascular hyporesponsiveness after hemorrhage was an artifact due to manipulation of the vessel for the blood flow measurements.

Statistical analysis

All data are presented as mean ± standard deviation (SD) of n observations. Vascular reactivity was defined as the maximum difference in MAP or blood flow in response to each infusion of NE and was calculated as a percentage of the vascular reactivity observed at baseline. In this study, the sources of variation are treatment (Phase I: sham, 60-min hemorrhage, 90-min hemorrhage; or Phase II: sham, hemorrhage, hemorrhage + NO inhibitor, and hemorrhage + ET antagonist), region (SMA, CA, LRA, and LFA), and time (baseline, 0, 1, 2, and 4 h). The analyzed measurements were MAP and blood flow. Statistical differences of the changes in MAP, blood flow, and the responses to NE were assessed by a three-factor ANOVA (treatment, region, and time) with repeated measurement on region and time, followed by post-hoc Tukey test. The comparisons of interest in Phase II were the following groups: hemorrhage versus sham hemorrhage (control), the L-NAME or PD-142893 versus hemorrhage, and L-NAME versus PD-142893. A $P < 0.05$ was considered significant.

RESULTS

General results

In Phase I, five of seven rats in the 60-min hemorrhage group survived more than 4 h after the end of the hypotensive period, one survived 3 h and 40 min, and one survived 3 h and 50 min. Only one of seven rats in the 90-min hemorrhage group survived the 4-h observation period, whereas the survival of the other six rats averaged 147 ± 60.4 min. The total blood loss in these groups was not significantly different (60 min: 19.7 ± 2.2 mL/kg vs. 90 min: 19.3 ± 2.5 mL/kg). Based on these observations and because of the goal to have the rats survive 4 h after the hypotensive period, subsequent studies used a 60-min hemorrhage. In the Phase II experiment, six of seven rats in the 60-min hemorrhage group survived the 4 h and one survived 3 h 10 min. All rats in the L-NAME and PD142893 pretreatment groups survived the 4 h. Blood loss in the L-NAME and PD-142893 groups was 24.7 ± 3.8 mL/kg vs. 22.5 ± 2.1 mL/kg, respectively). These higher blood volumes reflect the slight vasoconstrictor action observed after L-NAME or PD-142893 which resulted in the need to remove additional blood to maintain MAP at 50 mm Hg, but these volumes were not statistically higher than the other groups. Because the results of 60-min hemorrhage in both Phase I and II were similar, their results were combined for statistical comparisons.

Phase I: Effects of 60- versus 90-min hemorrhage

MAP and blood flow of the SMA, LRA, CA, and LFA in the sham hemorrhage group remained unchanged throughout the entire experimental period (Table 1). In this group, infusion of NE resulted in a 40.5 ± 4.5 mm Hg increase in MAP, and this relative change in MAP was taken as the 100% response for subsequent calculations. As shown in Figure 1, in the sham hemorrhage controls, the pressor response to NE on MAP at the other time points was similar to baseline.

The four arteries evaluated in this study had different responses to NE infusion. For example, in the SMA of the sham hemorrhage controls, blood flow at baseline decreased from 4.68 ± 0.70 mL/min to 1.32 ± 0.31 mL/min, corresponding to a −71.9 ± 6.5% change in blood flow after NE infusion. In the LRA, CA, and LFA of this group, blood flows at baseline decreased from 2.26 ± 0.77 to 1.03 ± 0.27 mL/min, from 3.76 ± 0.96 to 2.65 ± 0.06 mL/min and from 1.11 ± 0.63 to 0.80

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Table 1. **Pre-NE MAP and arterial blood flow after hemorrhagic shock in the rat**

	Baseline	0	1 h	2 h	4 h
MAP (mm Hg)					
Sham hemorrhage group (n = 7)	110.2 ± 7.97	111.8 ± 5.61	108.2 ± 5.51	109.4 ± 5.65	11.8 ± 4.79
60-min hemorrhage (n = 14)	112.0 ± 11.5	50.9 ± 1.31**	75.5 ± 18.0**	60.9 ± 15.5**	46.3 ± 12.2** (n = 11)
90-min hemorrhage (n = 7)	105.3 ± 5.50	49.7 ± 1.83**	48.3 ± 18.1**@@	38.95 ± 13.4**@@	30.4 (n = 1)
Blood flow of SMA (mL/min)					
Sham hemorrhage group (n = 7)	4.68 ± 0.70	4.55 ± 0.77	4.50 ± 0.62	4.60 ± 0.76	4.87 ± 0.92
60-hemorrhage (n = 14)	4.53 ± 1.61	1.63 ± 0.84**	2.47 ± 0.77**	1.76 ± 0.93**	1.31 ± 1.03** (n = 11)
90-min hemorrhage (n = 7)	4.61 ± 0.81	1.34 ± 0.41	1.42 ± 0.84**@	0.80 ± 0.53**	0.54 (n = 1)
Blood flow of LRA (mL/min)					
Sham hemorrhage group (n = 7)	2.26 ± 0.77	2.26 ± 0.71	2.15 ± 0.76	2.13 ± 0.79	2.24 ± 0.79
60-min hemorrhage (n = 14)	2.39 ± 0.66	0.42 ± 0.17**	1.13 ± 0.67**	0.74 ± 0.67**	0.52 ± 0.24** (n = 11)
90-min hemorrhage (n = 7)	2.16 ± 0.59	0.38 ± 0.15**	0.51 ± 0.24**	0.35 ± 0.17**	0.18 (n = 1)
Blood flow of CA (mL/min)					
Sham hemorrhage group (n = 7)	3.76 ± 0.96	3.67 ± 1.04	3.58 ± 1.04	3.53 ± 1.09	3.51 ± 1.21
60-min hemorrhage (n = 14)	3.15 ± 1.39	0.63 ± 0.30**	1.56 ± 1.21**	1.06 ± 1.14**	0.78 ± 0.49** (n = 11)
90-min hemorrhage (n = 7)	3.35 ± 0.83	0.42 ± 0.18**	0.46 ± 0.39**	0.28 ± 0.18**	0.25 (n = 1)
Blood flow of LFA (mL/min)					
Sham hemorrhage group (n = 7)	1.11 ± 0.63	1.07 ± 0.44	1.09 ± 0.37	1.01 ± 0.38	1.11 ± 0.48
60-min hemorrhage (n = 14)	1.20 ± 0.17	0.41 ± 0.18**	0.60 ± 0.23**	0.41 ± 0.22**	0.30 ± 0.21** (n = 11)
90-min hemorrhage (n = 7)	1.19 ± 0.34	0.30 ± 0.11**	0.34 ± 0.18**	0.29 ± 0.13**	0.16 (n = 1)

Mean ± SD; baseline, prehemorrhage; time 0, the end of the hypotensive period. $^{**}P < 0.01$ as compared with sham hemorrhage group, $@P < 0.05$, $@@P < 0.01$ as compared with 60-min hemorrhage group.

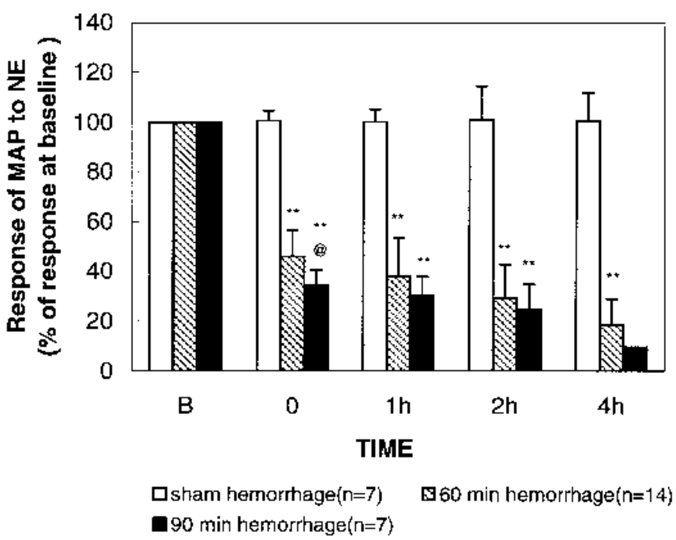

Fig. 1. **The changes of pressor response of MAP to NE (3 μg/kg, i.v.) after hemorrhagic hypotension at MAP of 50 mm Hg for 60 min or 90 min in the rat.** The increase of MAP after NE at baseline is considered as the 100% response. B, baseline; Time 0, the end of the hypotensive period. Shed blood was reinfused at the end of hypotensive period after time 0 measurement. Data represent mean ± SD. See Table 1 for n/ group. $^{**}P < 0.01$ as compared with sham hemorrhage, $^{@}P < 0.05$ as compared with 60-min hemorrhage group.

± 0.02 mL/min, respectively, corresponding to −54.6 ± 12.3%, −29.4 ± 1.7%, and −27.5 ± 2.1% changes in blood flows after NE infusion. As defined in the Materials and Methods section, these relative changes at baseline for each artery were assigned a value of 100% for subsequent calculations. Within an artery, there were no significant differences in the baseline response to NE infusion among the groups. In addition, as shown in Figure 2, for each artery the response of blood flow to NE in the sham hemorrhage group did not change from the baseline response during the experimental period.

Hemorrhagic hypotension at 50 mm Hg for 60 or 90 min induced an apparent loss of vascular reactivity to NE. The pressor response to NE on MAP in the 60-min hemorrhage group decreased to 45.9%, 37.8%, 29.2%, and 18.4% of the baseline pressor response (39.9 ± 5.0 mm Hg) at 0, 1, 2, and 4 h after the hypotensive period, whereas it was also significantly decreased over the 4-h period in the 90-min hemorrhage group (Fig. 1). These data were markedly different as compared with the sham hemorrhage group ($P < 0.01$).

The relative changes in blood flow at each time point after NE administration in the four arteries observed were significantly less than the baseline response after 60 min or 90 min of hemorrhagic hypotension (Fig. 2). For example, in the 60-min hemorrhage group, the blood flow response in the SMA after NE was reduced to 58.3% of the baseline response to NE at 0 h and to 13.6% at 4 h after the end of hypotension, whereas the LRA response was decreased to 52.3% at 0 h and to 10.5% at 4 h. The CA response decreased to 46.6% at 0 h and to 1.37% at 4 h, and the LFA response decreased to 30.3% at 0 h and to 0.77% at 4 h (Fig. 2). The responses at 2 h and 4 h were reduced more in the CA and LFA than in the SMA and LRA in the 60 min hemorrhage group ($P < 0.05$) (Fig. 2).

In the 90-min hemorrhage group, the reduced blood flow responses of these four arteries to NE were even more severe (Fig. 2). The blood flow of the LRA, CA, and LFA had no response to NE administration at 4 h in the one surviving rat.

Histologic evaluation of the four arteries examined in this study did not detect any affects of the anesthesia on endothelial integrity. In addition, no histologic evidence of mechanical damage to the endothelium was observed after either the 60 or 90 min of hemorrhagic hypotension in comparison with uninstrumented rats used as controls for this purpose.

Phase II: Effects of L-NAME and PD142893

In the 60-min hemorrhagic hypotensive rats pretreated with L-NAME (10 mg/kg) or PD142893 (0.1 mg/kg), MAP was higher at 1, 2, and 4 h as compared with the 60-min hemor-

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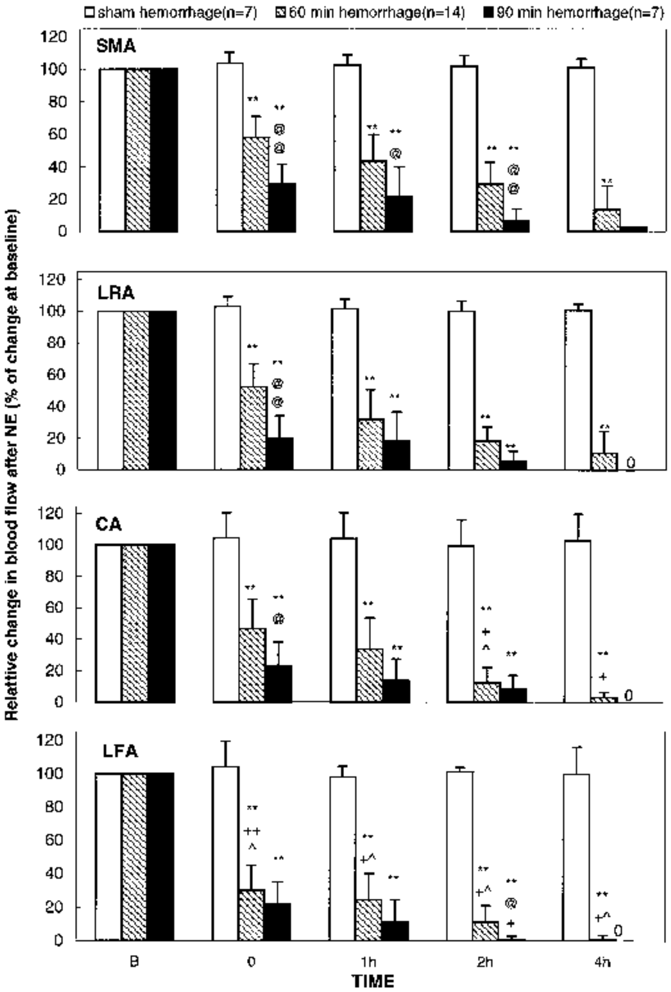

FIG. 2. **The blood flow responses of SMA, LRA, CA, and LFA to NE (3 μg/kg, i.v.) after hemorrhagic hypotension at MAP of 50 mm Hg for 60 min or 90 min in the rat.** The relative change in blood flow after NE administration at baseline was taken as the 100% response. B, baseline; Time 0, the end of the hypotensive period. Shed blood was reinfused at the end of the hypotensive period after the time 0 measurement. Data represent mean ± SD. See Table 1 for n/group. In the 90-min hemorrhage group, LRA, CA, and LFA had no responses to NE at 4 h. $^{**}P < 0.01$ as compared with sham hemorrhage group, $^{@}P < 0.05$, $^{@@}P < 0.01$ as compared with 60-min hemorrhage group, $^{+}P < 0.05$, $^{++}P < 0.01$ as compared with SMA, $^{\wedge}P < 0.05$, as compared with LRA.

rhage only group (Table 2). MAP peaked at 92.6 ± 4.2 mm Hg in the L-NAME group and peaked at 94.4 ± 10.7 mm Hg in the PD142893 group at 1 h ($P < 0.05$ from hemorrhage only group). Blood flow in the four arteries observed was generally lower after hemorrhage with L-NAME pretreatment as compared with the hemorrhage only group, and statistical significance was achieved in the SMA and LRA (Table 2). In contrast, the blood flow in the four arteries of PD142893 pretreated animals was similar to or slightly higher than that in the hemorrhage only group (Table 2). As a result, the blood flow of the observed arteries in the PD142893 group was significantly higher than in the L-NAME group (Table 2).

The pressor responses to NE on MAP in the L-NAME and PD142893 groups were significantly improved compared with the 60-min hemorrhage group at 0, 1, 2, and 4 h ($P < 0.01$, Fig. 3). For example, the pressor response on MAP maximally recovered at 1 h to 93.2% of the baseline pressor response (39.7 ± 2.6 mm Hg) in the L-NAME group and recovered to 82.7% of the baseline pressor response (39.7 ± 3.0 mm Hg) in the PD142893 group (Fig. 3).

The relative changes in blood flow of the four arteries to NE after L-NAME or PD142893 pretreatment were significantly higher than in the 60-min hemorrhage only group at all post-hemorrhage time points (Fig. 4). Their responses to NE were maximally recovered to 89.2% and 80.4% (SMA), 88.4% and 80.5% (LRA), 71.3% and 70.8% (CA), and 67.4% and 68.3% (LFA) of the baseline response in the L-NAME and PD142893 groups, respectively. Generally, as depicted in Figure 4, both L-NAME and PD142893 improved the contractile responses of SMA and LRA to NE better than CA and LFA, and improvement of contractility to NE in the LFA was better in the PD142893 than the L-NAME group at 2 and 4 h.

DISCUSSION

The occurrence of vascular hyporesponsiveness after hemorrhagic, endotoxic, or septic shock or associated with SIRS and MODS, has been well documented (1, 2, 4, 6). Many of these studies have investigated overall vascular reactivity based on changes in MAP or the responsiveness of a single blood vessel *in vitro*, with the aorta being the major blood vessel examined (2, 6, 16). Little is known, however, of the diversity of vascular reactivity *in vivo*, in different vascular beds following the above conditions. The present study was designed to investigate this issue in four different vasculatures, which supply different organ systems in a rat model of hemorrhagic shock.

The results of the present study indicated that based on changes in MAP, 60 min or 90 min of hemorrhagic hypotension induced an apparent loss of pressor response to NE, as well as a decrease in the contractile response of individual arteries to NE, in agreement with the observations of Thiemermann et al. (23) that vascular hyporesponsiveness occurred within 2 h after hemorrhage. The present study also observed that the decrease in blood flow and the contractile response to NE of the four arteries was slightly more severe after 90 min of hypotension than 60 min. The 60-min hypotensive insult caused over a 50% and 80% loss of the pressor response to NE on MAP at 0 and 4 h after the end of the hypotensive period, respectively, whereas 90 min of hypotension caused over 65% and 90% loss of responsiveness at these times. In addition, the responses of the four arteries studied to NE after the hemorrhage insult were not the same. Generally, the CA and FLA beds appeared to be more sensitive to the hemorrhage insult than the SMA and LRA.

Vascular tone and its contraction/dilation are regulated by a complicated system, including neural and humoral factors during various stresses. As mentioned above, many factors may interfere with the vascular reactivity following shock. The sympathetic-adrenergic receptor system (9, 27), the neuroendocrine system (14), mediators such as NO, ET, and cytokines such as TNF-α and IL-1 (3, 5, 10–17, 23) have been proposed as major contributors to altered vascular responsiveness observed following shock.

The exact mechanisms for the differential response to NE in the four arteries studied are unknown. However, Thiemermann

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Table 2. **Effects of pretreatment with L-NAME and PD142893 on pre-NE MAP and blood flow after hemorrhagic shock in the rat**

	Baseline	0	1 h	2 h	4 h
MAP (mm Hg)					
Sham hemorrhage group (n = 7)	110.2 ± 7.97	111.8 ± 5.61	108.2 ± 5.51	109.4 ± 5.65	11.8 ± 4.79
60-min hemorrhage (n = 14)	112.0 ± 11.5	50.9 ± 1.31**	75.5 ± 18.0**	60.9 ± 15.5**	46.3 ± 12.2** (n = 11)
L-NAME group (n = 7)	110.6 ± 5.74	50.8 ± 0.78**	92.6 ± 4.18@	72.7 ± 9.15**	55.6 ± 5.05**
PD142893 group (n = 7)	111.4 ± 6.17	50.4 ± 1.30**	94.4 ± 10.7@	82.9 ± 18.6**@@	65.0 ± 24.6**@
Blood flow of SMA (mL/min)					
Sham hemorrhage group (n = 7)	4.68 ± 0.70	4.55 ± 0.77	4.50 ± 0.62	4.60 ± 0.76	4.87 ± 0.92
60-hemorrhage (n = 14)	4.53 ± 1.61	1.63 ± 0.84**	2.47 ± 0.77**	1.76 ± 0.93**	1.31 ± 1.03** (n = 11)
L-NAME group (n = 7)	5.02 ± 2.13	1.36 ± 0.51**	1.41 ± 0.67**@	1.09 ± 0.63**	0.87 ± 0.36**
PD142893 group (n = 7)	4.84 ± 0.41	1.79 ± 0.71**	2.78 ± 0.83**^^	2.51 ± 0.64**^	2.05 ± 0.86**
Blood flow of LRA (mL/min)					
Sham hemorrhage group (n = 7)	2.26 ± 0.77	2.26 ± 0.71	2.15 ± 0.76	2.13 ± 0.79	2.24 ± 0.79
60-min hemorrhage (n = 14)	2.39 ± 0.66	0.42 ± 0.17**	1.13 ± 0.67**	0.74 ± 0.67**	0.52 ± 0.24** (n = 11)
L-NAME group (n = 7)	2.13 ± 0.32	0.38 ± 0.11**	0.35 ± 0.11**@	0.29 ± 0.08**@	0.26 ± 0.07**
PD142893 group (n = 7)	2.16 ± 0.61	0.45 ± 0.09**	1.13 ± 0.63*	0.78 ± 0.39**^	0.50 ± 0.20**
Blood flow of CA (mL/min)					
Sham hemorrhage group (n = 7)	3.76 ± 0.96	3.67 ± 1.04	3.58 ± 1.04	3.53 ± 1.09	3.51 ± 1.21
60-min hemorrhage (n = 14)	3.15 ± 1.39	0.63 ± 0.30**	1.56 ± 1.21**	1.06 ± 1.14**	0.78 ± 0.49** (n = 11)
L-NAME group (n = 7)	3.83 ± 0.95	0.53 ± 0.23**	0.89 ± 0.50**	0.56 ± 0.31**	0.44 ± 0.22**
PD142893 group (n = 7)	4.03 ± 0.26	0.76 ± 0.29**	2.13 ± 0.71*^	1.37 ± 0.87**	0.98 ± 0.67**
Blood flow of LFA (mL/min)					
Sham hemorrhage group (n = 7)	1.11 ± 0.63	1.07 ± 0.44	1.09 ± 0.37	1.01 ± 0.38	1.11 ± 0.48
60-min hemorrhage (n = 14)	1.20 ± 0.17	0.41 ± 0.18**	0.64 ± 0.23**	0.41 ± 0.22**	0.30 ± 0.21** (n = 11)
L-NAME group (n = 7)	1.11 ± 0.33	0.30 ± 0.13**	0.38 ± 0.21**	0.27 ± 0.09**	0.26 ± 0.09**
PD142893 group (n = 7)	1.31 ± 0.13	0.36 ± 0.09**	0.77 ± 0.26^	0.54 ± 0.20**^	0.38 ± 0.20**

Mean ± SD; time 0, the end of the hypotensive period. *P < 0.05, **P < 0.01 as compared with sham hemorrhage group, @P < 0.05, @@P < 0.01 as compared with 60-min hemorrhage group, ^P < 0.01, ^^P < 0.01 as compared with L-NAME group.

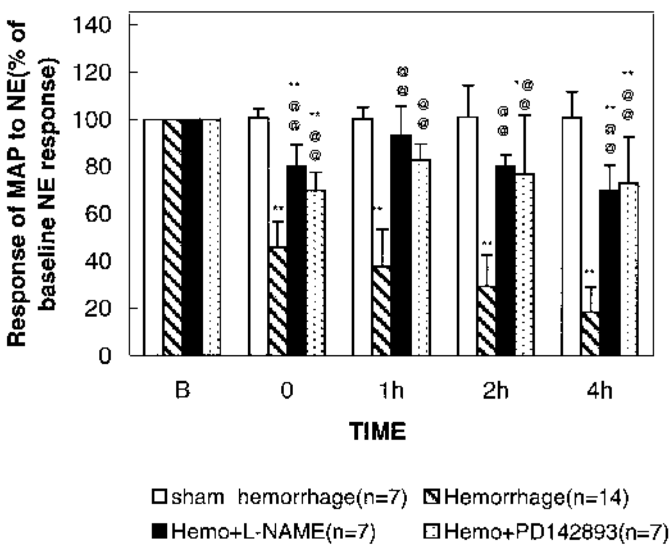

Fig. 3. **Effects of pretreatment with L-NAME and PD142893 on the pressor response of MAP to NE after hemorrhage shock in the rat.** L-NAME (10 mg/kg, i.v.) and PD142893 (0.1 mg/kg) were given 15 min before the end of the hypotensive period. Shed blood was reinfused at the end of the hypotensive period after the time 0 measurement. The increase of MAP after NE at baseline is considered as the 100% response. B, baseline; Time 0, the end of the hypotensive period. Data represent mean ± SD. *P < 0.05, **P < 0.01 as compared with sham hemorrhage group, @@P < 0.01 as compared with 60-min hemorrhage group.

et al. (23) reported that the inducible NOS (iNOS) activity in lung, liver, and spleen was significantly increased 150 min and 330 min following hemorrhagic shock, and that liver and spleen iNOS activity was significantly higher than that in mesentery and kidney at these times. In addition, ET-1, constitutive NOS (cNOS), TNF-α, and IL-1β reportedly had differential expression in various organs during shock (28). The roles of NO in different blood vessels and the diversity of the expression and secretion of some cytokines that affect vascular reactivity in different organs (28) might be an important reason for the difference in vascular hyporeactivity among the four arteries observed in the present study. However, none of these mediators were measured in the present experiment and such measurements should be included in subsequent investigations.

As mentioned, the role of NO in vascular hyporeactivity has been well documented (3, 5, 6, 10–13, 23). ET has also been implicated in the pathophysiology and pathogenesis of circulatory shock, but its role in vascular hyporesponsiveness during shock has not been determined. The present experiment indicated that the pretreatment with the nonselective NOS inhibitor L-NAME or nonselective endothelin receptor antagonist PD142893 prevented, to a degree, the decreased vascular reactivity induced by hemorrhagic shock. L-NAME pretreatment recovered the vascular reactivity of SMA, LRA, CA, and LFA to 89.2%, 88.4%, 71.3%, and 67.4% of baseline level, respectively, whereas PD142893 restored the vascular reactivity to 80.4%, 80.5%, 70.8%, and 68.3% of baseline level, respectively. Both L-NAME and PD142893 improved the vascular reactivity of SMA and LRA better than that of CA and LFA. These results suggest that NO and ET, although reported to have opposite effects on vascular tone (29–31), appeared to have similar effects on vascular hyporeactivity after hemorrhagic shock. Although the mechanisms responsible for these observations are not known, it has been reported that ET could induce the release of NO and cyclooxygenase products, which have been found to be involved in the modulation of vascular reactivity (20, 26, 30–32). In addition, NO may modify endo-

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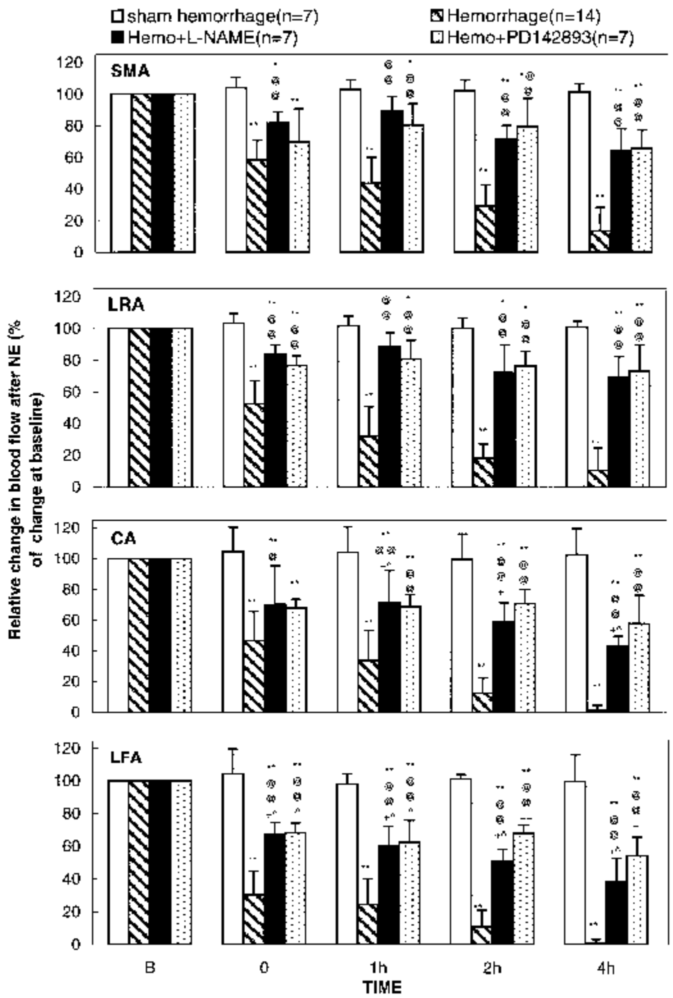

FIG. 4. **Effects of pretreatment with L-NAME and PD142893 on the blood flow responses of SMA, LRA, CA, and LFA to NE (3 μg/kg, i.v.) after hemorrhagic shock in the rat.** L-NAME (10 mg/kg, i.v.) and PD142893 (0.1 mg/kg) were given 15 min before the end of the hypotensive period. Shed blood was reinfused at the end of the hypotensive period after the time 0 measurement. The relative change in blood flow after NE administration at baseline was taken as the 100% response. B, baseline; Time 0, the end of the hypotensive period. Data represent mean ± SD. **P* < 0.05, ***P* < 0.01 as compared with sham hemorrhage group, @*P* < 0.05, @@*P* < 0.01 as compared with 60 min hemorrhage group, ~*P* < 0.05, ~~*P* < 0.01 as compared with L-NAME group, +*P* < 0.05 as compared with SMA, ^*P* < 0.05 as compared with LRA.

thelin receptor binding (33). The doses of L-NAME and PD142893 used in the present study were selected from the literature as effective to inhibit the synthesis of NO or block the effects of ET-1 (25, 26). Further studies are needed to ascertain the actual mechanisms for the effects of NO and ET inhibition observed in our study, to determine whether effects would be dose dependent or whether these inhibitors would be effective if given even later after the induction of hemorrhagic shock.

It should be noted that despite the similar response of the four blood vessels to NE in the L-NAME- and PD-142893–treated animals in the present study, differences existed in the actions of these agents. For example, L-NAME treatment tended to reduce blood flow to the vessels studied in comparison to the hemorrhage only group, whereas PD-142893 treatment may have increased blood flow to these vessels. Although improvement in vascular reactivity to NE after L-NAME treatment would suggest a beneficial effect, reduced blood flow could worsen ischemic injury after hemorrhage. NO inhibition has been shown to improve survival after hemorrhagic shock (25). Because NO may have numerous functions in the pathophysiology of hemorrhagic shock (11), it is possible that better targeted NO inhibition could overcome the potential detrimental effects of reduced blood flow to vital organs and together with the improvement in vascular reactivity, serve as improved treatment of hemorrhagic shock.

In the present study, we have referred to the observations on the blood flow response to NE in the four vasculatures examined, as changes in vascular responsiveness, suggesting effects on vascular tone. It could be argued that other events occurring after NE infusion could be influencing the results observed that may be independent of vascular tone. For example, the direct effects of NE on the heart, increasing stroke volume, could lead to pressure-driven blood flow changes to explain the data obtained. However, in the present study, blood flow decreased rather than increased after NE infusion. In addition, NE has minimal β-2 adrenergic receptor activity, so it is unlikely that the decreased blood flow observed is a consequence of vasodilation. However, we realize that our data only indirectly reflect vascular reactivity.

CONCLUSION

The present study showed that hemorrhagic hypotension at 50 mm Hg for 60 and 90 min resulted in an apparent loss of vascular reactivity to NE that could not be attributed to mechanical damage to the endothelium. Hemorrhagic hypotension for 90 min resulted in almost an entire loss of vascular reactivity to NE by the end of the 4-h observation period. Nevertheless, from the improvement in vascular responsiveness observed in the present study after NO and ET inhibition, it would seem that the production of these mediators, as well as the generation of oxygen free radicals and other inflammatory effectors resulting from reduced tissue perfusion and subsequent tissue ischemia, may also be involved in the loss of vascular responsiveness following shock (34, 35). In addition, different vasculatures did not respond the same to the hemorrhage insult. The response was more severe in CA and LFA than in SMA and LRA. Additional studies are warranted to determine the mechanisms responsible for the similar improvement on vascular reactivity observed after NO or ET inhibition after hemorrhagic shock.

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